On the Development of *Artemia salina* L. (Crustacea: Anostraca)[1]

BY

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(*With a plate*)

INTRODUCTION

Previous work on the development of Anostracans, apart from early observations reported by Claus (1885) comprise Cannon's (1924) brief account of the development of the fairy shrimp, *Chirocephalus diaphanus* and Heath's (1924) extensive account of the development of Anostracans. Weisz (1946), who was primarily interested in segment formation in relation to size and shape, described growth rate in *A. salina*. Hall (1959a, 1959b) published brief accounts of the development of the eggs of *Chirocephalus* in relation to depth of water and low temperature. Pai (1958) described the post-embryonic stages of the phyllopod crustaceans, *Triops* (*Apus*), *Streptocephalus*, and *Estheria*. Recently Prophet (1963) studied the influence of temperature, drying, dilution of the culture medium, egg age, etc. on the hatching of Anostracan eggs. This paper is an account of the development of *Artemia salina*.

METHODS

Artemia eggs were collected from Sambhar Salt Lake, Rajasthan. The eggs were kept in the laboratory in lake water of different salinities to study the relation between hatching time and salinity. Yeast pellets were given as food to the developing nauplii. The larvae were fixed in Bouin's fluid at intervals and stained with borax carmine. The observations recorded in this paper are from whole mounts as well as from living specimens from the aquaria.

[1] This work was carried out in the Department of Zoology, University of Rajasthan, Jaipur (India).

Observations

The eggs are spherical, hard-shelled, brown-coloured, often floating on the surface in long interlocking filaments. Each egg measures 0·25-0·30 mm. in diameter (Plate, 1). Drying was not necessary for hatching; eggs kept at 60° C. for 48 hours hatched quickly.

Stage 1: hatching to 18 hours; (Plate, 2-4).

The larva at this stage is a nauplius. Initially, it is 0·2 mm. long with broad head, three pairs of cephalic appendages (antennules, antennae, and mandibles), and an unsegmented trunk. The head is distinguished from the trunk region by a constriction visible from the ventral side, and also by the nuchal organ on the dorsal side. As in other branchiopods, the antennules are uniramous and unsegmented, and bear only two terminal setae. The antennae comprise a sympod of two segments and an unsegmented simple exopod and endopod. The first segment bears at its distal end a stout bifid curved spine which is frequently referred to as a masticatory process. The bifid point of this spine lies just behind the mouth, between the long labrum and the ventral side of the body. The second segment bears a single posterior seta at its distal end. The seta is long and its distal half has no setule as reported by Heath (1924). The exopod bears two long terminal setae and a row of seven long ventral setae with no setules but with a distinct hinge at about their midpoint. The endopod, sharply separated from the exopod, is quite short, only one-third of the length of the exopod, and bears four long terminal setae of the same form and about as long as the ventral setae of the exopod. The mandibles are uniramous and about as long as the sympod of the antenna. Its segmentation is not clearly marked but it appears to comprise a large basal plate (sympod) and a short terminal joint (endopod). There are six setae in all, a terminal group of three rather short smooth setae and three stout setulose spines on the inner side of the sympod. As the two mandibles work more or less in a plane parallel to the ventral surface of the body, the inner spines of each approximately oppose those of the other. The labrum is very large lying against the ventral surface. It overlaps the mandibles completely. The median eye (ocellus) is a tiny pigmented spot in between the two compound eyes, which are not pigmented. The dorsal or nuchal organ, highly swollen up with yolky material, is found below the eyes.

In the later changes the trunk grows more slender. The rudiments of the maxilla, maxillulae, and the first four swimming appendages are visible through the delicate cuticle. The larvae at this stage are 0·4 mm. long and have commenced to feed.

Stage 2: 18+ to 42 hours; (Plate, 5).

The larva at this stage measures 0·49-0·69 mm. (average of 34 specimens 0·61 mm.). The three pairs of cephalic appendages are as in stage 1 but the cilia are long and tapering. The posterior end of the trunk shows a rudiment of the caudal cerci. The maxillae and maxillulae have essentially the same form that they have in later stages. Each maxilla consists of a basal portion, the protopodite, to which a slender appendage is attached latero-posteriorly. Each maxillula is little more than a low conical elevation rising immediately behind the maxilla and is usually inclined towards the mid-line. The free end of the maxillary palp carries two delicate setae. The median eye becomes more pigmented and is very conspicuous at this stage. Each compound eye becomes enlarged and migrates away from the median eye. The dorsal organ occupies a larger portion of the cephalic region and does not show any change in shape. Ventrally the labrum covers the mouth. All the appendages seem to rise from the mid-ventral line of the body. The trunk region shows as many as five segments.

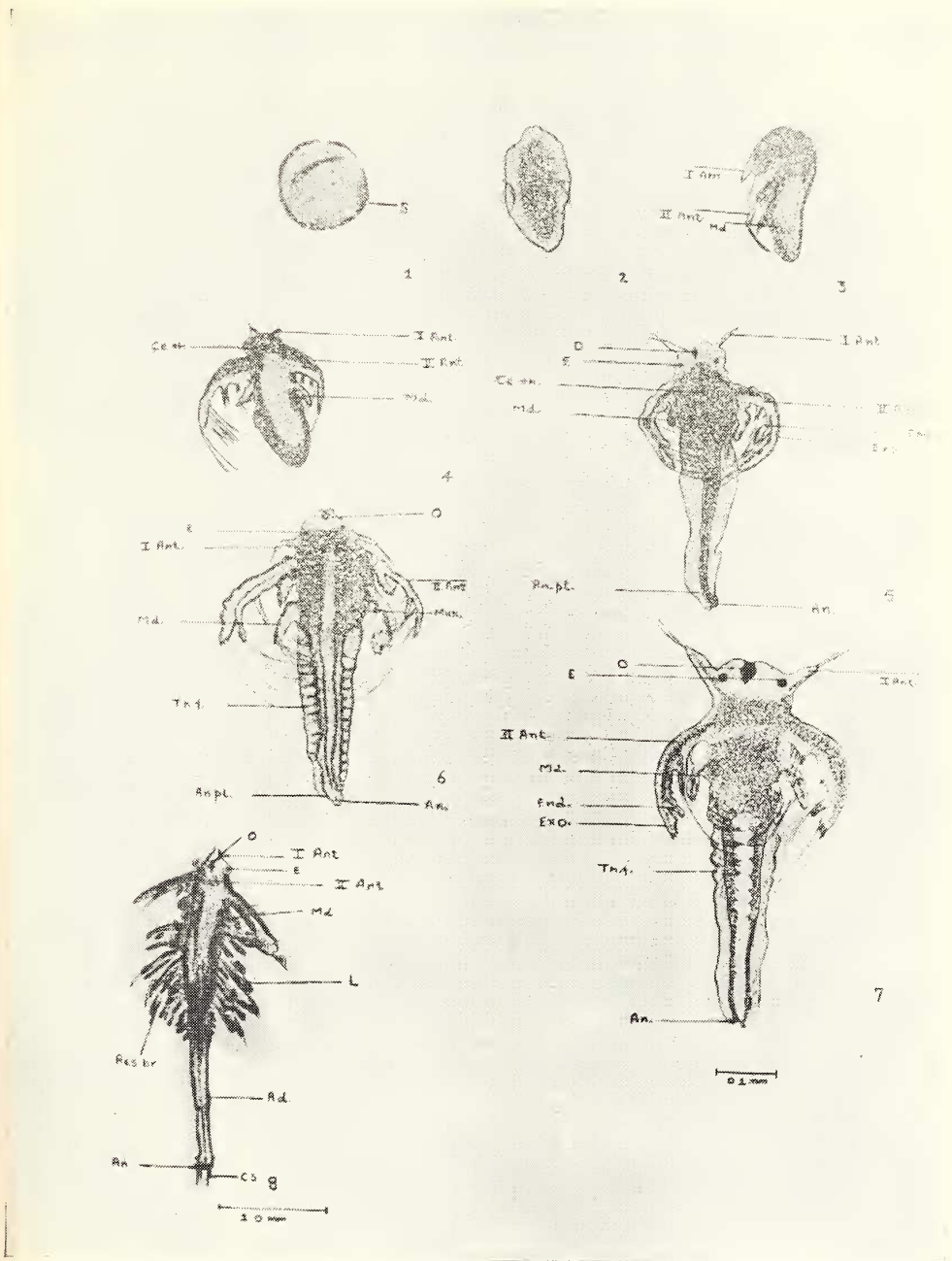
The later changes involve an increase in the length of the maxillary region, and of the antennae and mandibles. The compound eyes are as prominent as the median eye.

Stage 3: 42+ to 66 hours; (Plate, 6).

The larva is now 1·2 mm. long. The antennule is short, straight, unsegmented, with two bristles. The antennae and mandibles have increased in length. The endopodite of the antenna is thumb-like and is supplied with long setae. The maxillary region is much larger since the maxillary gland develops in the larval stages. In the trunk region nine buds of appendages having flagella protrude, the first six showing more or less clearly defined endites. Posterior to this the buds become less conspicuous and finally there is an unsegmented part. The alimentary canal is seen with two digestive pouches below the transparent exoskeleton and the food is visible in the form of suspended particles.

Stage 4: 66+ to 96 hours; (Plate, 7).

The larva is *c* 2·6 mm. long. The compound eyes are elevated above the cephalic region and are borne on stalks. The antennae and mandibles become more ventral in position. The mandibles are now in the form of a pouch. The maxillary glands are well developed below the mandibles. Eleven pairs of thoracic appendages are seen, the first four with endites and the other seven in the form



Stages in the development of *Artemia salina* L.

Fig. 1. Egg ; Figs. 2-8. Larva at different stages (in hours from hatching) ; Figs. 2-4. hatching to 18 hrs. ; Fig. 5. 18+ to 42 hrs. ; Fig. 6. 42 to 66 hrs. ; Fig. 7. 66+ to 96 hrs. ; Fig. 8. 96+ to 144 hrs.

Ad. Abdomen ; An. Anus ; An.pl. Anal plate ; I Ant. Antennule ; II Ant. Antenna ; Ce.th. Cephalothorax ; C.S. Caudal setae ; E. Eye ; End. Endopodite ; Exo. Exopodite ; L. Leg ; Md. Mandible ; Max. Maxilla ; O. Median eye ; Res.br. Respiratory bract ; S. Shell ; Th.f. Thoracic fold.

of protuberances. The bracts of the first four limbs are clearly formed. The flabella are also distinctly formed and show clear demarcation from the last endite at one end to the bract at the other. The thoracic and abdominal regions are more clearly marked than in the previous stages. The abdominal region has no appendage.

Stage 5: 96+ to 144 hours; (Plate, 8).

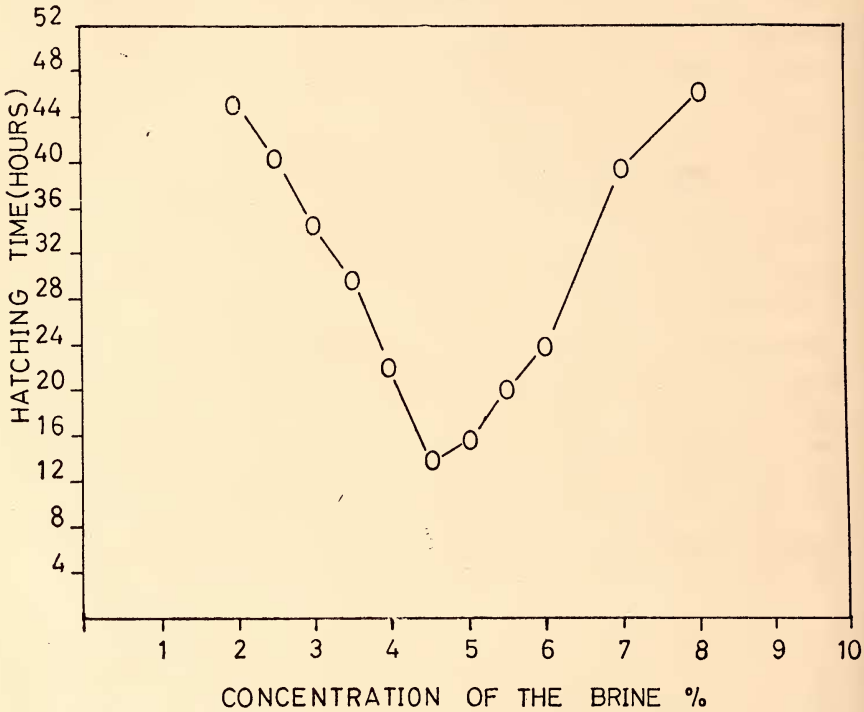
The larva is 4·5 mm. long. The appendages of the thoracic region are very well developed and are followed by seven abdominal segments without appendages. The first nine appendages of the thorax have well marked endites, flabella, and bracts. The first limb appears distinctly smaller than the second limb. The antenna at this stage is completely rotated on the ventral side and is very much reduced, thereby indicating the female sex. In the female, a thickening in the form of a rounded elevation is developed around the posterior region of 11th thoracic segment and first and second abdominal segments which form the egg pouch. The caudal furcae are elongated and have nine setae. The frontal organs have become plumose and sensory. The larva is almost an adult now. In the later stages the larva grows in size.

Discussion

The newly hatched larva is a true nauplius without any segmentation in the trunk region but possesses a median eye in the anterior part of the head and three pairs of cephalic appendages. The larvae show phototropic movements and are found at the surface feeding on the scum deposited on the sides of the aquarium. Their number is more on sides which are towards light. This observation is true when the depth of water in the container is more than 6 cm. otherwise they are equally distributed. The time taken for hatching in different salinities is shown in the text-figure below. It shows that 4·5% salinity with a hatching time of 12 hours is the most suitable concentration. The hatching time for mud containing eggs is about 24-48 hours after immersion. Most workers (Heath 1924, Pai 1958) have obtained nauplius larvae in 24-48 hours. The larvae also hatch out more quickly in summer than in winter. In winter hatching is delayed by 12 hours.

The larva of *Artemia* is easily differentiated from other branchiopod nauplii immediately at hatching. It has no carapace, no segmentation in the post-mandibular region, lacks two anal setae but has three

pairs of cephalic appendages, and has a prominent sessile median eye in the notch in the anterior part of the head.



Text-figure. Relationship between hatching time of *Artemia salina* eggs and the salinity of the medium.

Segmentation and appendages

External appearance of segmentation is noticeable 22 hours after hatching when, along with trunk segments, 4-5 buds of appendages of the trunk are seen to protrude. The first thoracic appendage with its endites is seen in the larvae of 40-48 hours after hatching, and the distal endite of this appendage develops into a respiratory bract. Lankester (1881) stated that the epipodites are richly supplied with blood and he called them 'branchiae'. In the present study on *Artemia*, it is found that the number of segments has no relation with the number of appendages and further the number of segments is more than the number of appendages of the adult. Linder (1952) elucidated the relationship between the number of segments, the number of appendages, and the number of posterior apodous segments, while working on the North American Notostraca. According to him there is no correlation between the number of appendages and the segments bearing them.

The three cephalic appendages, viz. antennules, antennae, and mandibles, show interesting variation in the phyllopods. In *Artemia*, the antennules are uniramous and unsegmented, and have two bristles at the free end. The antennae and mandibles are strong and biramous, and carry long setae. Setules as found in the larvae of other Anostracans (Heath 1924, Pai 1958) are absent. Later, these appendages undergo reduction. The antennae are small in the female. This observation could not be made in the male, since the eggs are parthenogenetic. The mandible loses its biramous character and develops into a cup-shaped structure with spines. The first trunk appendage by this time is fully developed and is provided with endites. It functions as a swimming appendage.

Development of appendage

Each swimming appendage appears in the form of a low ridge (Plate, 6) when viewed posteriorly and totally lacks the characteristic divisions of the fully developed organ. This is the typical condition of the seventh body segment in stage 3. The appendages anterior to it show four sub-divisions. Along the dorsal border in contact with the body proper are branchial lobes (Linder 1952, called them praepipodite and epipodite). The branchial section is separated by a distinct notch from the slightly differentiated flabellum (Linder 1952, called it exopodite) whose limit more ventrally is indicated by a cleft adjacent to the region of the future endites. The endites comprising somewhat the larger portion of the appendage bear one small notch which marks the outer limit of the gnathobase. The two branchial lobes are clearly defined and are sharply separated by a comparatively deep cleft from the flabellum. Each of the six endites is formed on the inner side of the appendage. The early appearance of the flabellum, its position with reference to the principal axis of the appendage, and the fact that it bears one to two small setae corresponding to those on 6 endites, suggest that the flabellum is an exopodial structure and the endites endopodial as Huxley and others suggested long ago.

Eye and Maxillary gland

The median pigmented nauplius eye is visible in the early larval stage (stage 1) in a notch in the anterior part of the head. It persists up to 48 hours. In stage 2 both the median eyes and paired sessile eyes are seen but, faintly, in larvae of 96 hours the paired eyes are borne on stalks. The maxillary gland is found below the second maxilla and appears in a 30-hour old larva. It becomes conspicuous in 66 hours.